CLAIMS

WHAT IS CLAIMED:

1. A chimerical peptide-nucleic acid fragment comprising:

(a) a cell-specific, compartment-specific or membrane-specific signal peptide, with the exception of a KDEL signal sequence,

(b) a linkage agent,

(c) a nucleic acid (oligonucleotide),

the signal peptide being linked via the linkage agent which via amino acids at the carboxy-terminal end of the signal peptide is linked therewith so as to ensure the appropriate nucleic acid introduction into cell organelles and cells.

2. The chimerical peptide-nucleic acid fragment according to claim 1, characterized in that the nucleic acid consists of at least two bases.

3. The chimerical peptide-nucleic acid fragment according to any one of claims 1 to 2, characterized in that the nucleic acid has a secondary structure.

4. The chimerical peptide-nucleic acid fragment according to any one of claims 1 to 3, characterized in that the nucleic acid has a palindromic sequence.

5. The chimerical peptide-nucleic acid fragment according to claim 4, characterized in that the nucleic acid may form a "hairpin loop".

6. The chimerical peptide-nucleic acid fragment according to claim 5, characterized in that the nucleic acid may hybridize with itself and may form an overhanging 3' end or 5' end ('sticky end').

7. The chimerical peptide-nucleic acid fragment according to any one of claims 1 to 6, characterized in that the nucleic acid is a ribonucleic acid, preferably a deoxyribonucleic acid.

8. The chimerical peptide-nucleic acid fragment according to claim 7, characterized in that the nucleic acid has chemically modified 'phosphorus thioate' linkages.

9. The chimerical peptide-nucleic acid fragment according to any one of claims 1 to 8, characterized in that the nucleic acid carries a reactive linkage group.

10. The chimerical peptide-nucleic acid fragment according to claim 9, characterized in that the reactive linkage group contains an amino function when the linkage agent contains an amino-reactive grouping.

11. The chimerical peptide-nucleic acid fragment according to claim 9, characterized in that the reactive linkage group contains a thiol function when the linkage agent contains a thiol-reactive grouping.

12. The chimerical peptide-nucleic acid fragment according to claim 10 or 11, characterized in that the linkage grouping present is bound to the nucleic acid via at least one C2 spacer, but preferably one C6 spacer.

13. The chimerical peptide-nucleic acid fragment according to claim 12, characterized in that the linkage grouping is localized at the 3' hydroxy/phosphate terminus or at the 5' hydroxy/phosphate terminus of the nucleic acid, but preferably at the base.

14. The chimerical peptide-nucleic acid fragment according to any one of claims 10 to 13, characterized in that defined nucleic acids, antisense oligonucleotides, messenger RNAs or transcribable and/or replicatable genes are linked with the 5' end and/or 3' end.

15. The chimerical peptide-nucleic acid fragment according to claim 14, characterized in that the nucleic acid to be linked contains chemically modified 'phosphorus thioate' linkages.

16. The chimerical peptide-nucleic acid fragment according to claim 14 to 15, characterized in that the gene be linked contains a promotor, preferably a mitochondrial promoter.

17. The chimerical peptide-nucleic acid fragment according to any one of claims 1 to 16, characterized in that the signal peptide has a reactive amino acid at the carboxy-terminal end, preferably a lysine or cysteine, when the linkage agent contains an amino-reactive or thiol-reactive grouping.

18. The chimerical peptide-nucleic acid fragment according to any one of claims 1 to 17, characterized in that the signal peptide carries a cell-specific, compartment-specific or membrane-specific recognition signal.

19. The chimerical peptide-nucleic acid fragment according to any one of claims 1 to 18, characterized in that the signal peptide has a cell-specific, compartment-specific or membrane-specific peptidase cleavage site.

20. The chimerical peptide-nucleic acid fragment according to any one of claims 1 to 19, characterized in that the peptide consists of the compartment-specific cleavable signal peptide of the human mitochondrial ornithine transcarbamylase, extended by an artificial cysteine at the C terminus.

21. The chimerical peptide-nucleic acid fragment according to any one of claims 1 to 20, characterized in that the linkage agent is a bifunctional, preferably heterobifunctional cross-linker.

22. The chimerical peptide-nucleic acid fragment according to any one of claims 1 to 21, characterized in that the linkage agent contains thiol-reactive and/or amino-reactive groupings when the signal peptide and the nucleic acid carry thiol and/or amino groups as linkage sites.

23. The chimerical peptide-nucleic acid fragment according to any one of claims 1 to 22, characterized in that the linkage agent is m-maleimido-benzoyl-N-hydroxy-succinimide ester or a derivative thereof.

24. The chimerical peptide-nucleic acid fragment according to any one of claims 1 to 23, characterized in that the molecule can overcome membranes with and without membrane potential by utilizing natural transport mechanisms.

25. The chimerical peptide-nucleic acid fragment in the form of a linear-cyclic plasmid, characterized in that the plasmid comprises at least one replication origin and that both ends of the nucleic acid portion are cyclized, at least one cyclic end having a modified nucleotide which via a linkage agent can be liked with a cell-specific, compartment-specific or membrane-specific signal peptide.

26. The chimerical peptide-nucleic acid fragment according to claim 25, characterized in that the nucleic acid portion further comprises at least one promoter, preferably a mitochondrial promoter, especially preferably the mitochondrial promoter of the light strand.

27. The chimerical peptide-nucleic acid fragment according to any one of claims 25 and 26, characterized in that the nucleic acid portion further comprises transcription-regulatory sequences, preferably mitochondrial transcription-regulatory sequences.

28. The chimerical peptide-nucleic acid fragment according to any one of Claims 25-27, characterized in that the transcription-regulatory sequences have at least one binding site of a transcription initiation factor.

29. The chimerical peptide-nucleic acid fragment according to any one of Claims 25 to 28, characterized in that the transcription-regulatory sequences have at least one binding site for the RNA synthesis apparatus, preferably the binding site for the mitochondrial transcription factor 1 and the mitochondrial RNA polymerase.

30. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 29, characterized in that the transcription-regulatory sequences are arranged in the 3' direction of the promoter.

31. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 30, characterized in that the transcription is regulated by elements of the mitochondrial H-strand and L-strand transcription control.

32. The chimerical peptide-nucleic acid fragment according to claim 31, characterized in that what is called 'conserved-sequence-blocks' of L-strand transcription act as transcription control elements.

33. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 32, characterized in that the plasmid further comprises at least one transcription termination site.

34. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 33, characterized in that the transcription termination site has a binding sequence of a mitochondrial transcription termination factor.

35. The chimerical peptide-nucleic acid fragment according to claim 34, characterized in that the transcription termination site has the binding sequence of a preferably bidirectionally acting transcription termination factor.

36. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 35, characterized in that the replication origin is a mitochondrial replication origin, preferably the replication origin of the heavy mtDNA strand having at least one 'conserved sequence block'.

37. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 36, characterized in that the plasmid further comprises at least one regulatory sequence for the replication.

38. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 37, characterized in that the regulatory sequence for the replication is a mitochondrial sequence motif.

39. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 38, characterized in that the plasmid further comprises a selection gene, preferably an antibiotic-resistance gene, preferably the oligomycin - or chloramphenicol - resistance gene.

40. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 39, characterized in that the plasmid further contains a multiple cloning site which permits the expression of 'foreign genes'.

41. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 40, characterized in that the multiple cloning site comprises recognition sequences for restriction endonucleases which do preferably not occur in another site of the plasmid.

42. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 41, characterized in that the multiple cloning site is arranged in the 3' direction of the promoter and in the 5' direction of the transcription termination site.

43. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 42, characterized in that the multiple cloning site is arranged in the 5' direction of the selection gene.

44. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 43, characterized in that the nucleic acid fragment has (phosphorylated) ends capable of litigation.

45. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 44, characterized in that the nucleic acid fragment has 'blunt ends' or overhanging 3' ends, preferably overhanging 5' ends.

46. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 45, characterized in that the nucleic acid fragment has 4 nucleotides comprising 5' overhangs which do not have a self-homology (palindromic sequence) and are not complementary to one another either.

47. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 46, characterized in that the ends of the nucleic acid fragment are cyclized via synthetic oligonucleotides.

48. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 47, characterized in that the overhanging 5' ends of the two oligonucleotides are complementary to one differing end of the nucleic acid each.

49. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 48, characterized in that two differing 'hairpin loops' are used for the cyclization, one being specific (complementary) to the 'left' plasmid end and the other being specific to the 'right' plasmid end of the nucleic acid.

50. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 49, characterized in that the modified nucleotide is localized preferably within the 'loop'.

51. The chimerical peptide-nucleic acid fragment according to any one of claims 25 to 50, characterized in that the plasmid DNA is amplified enzymatically by suitable oligonucleotides which have at least one recognition sequence for a restriction endonuclease which occurs preferably in non-repeated fashion in the plasmid sequence.

52. The chimerical peptide-nucleic acid fragment according to claim 51, characterized in that the restriction endonuclease to be used generated overhanging ends, preferably 5' overhanging ends, the cleavage site being localized preferably outside the recognition sequence.

53. The chimerical peptide-nucleic acid fragment according to claim 51 or 52, characterized in that the restriction endonuclease is B*sa*I.

54. A process for the production of a chimerical peptide-nucleic acid fragment according to any one of claims 1 to 53, characterized by the following stages:

(a) reaction of a nucleic acid (oligonucleotide) containing a functional linkage group having a linkage agent,

(b) reaction of the construct of (a) with amino acids at the carboxy-terminal end of a peptide, containing a signal sequence, with the exception of a KDEL signal sequence, and

(c) optionally extension of the chimerical peptide-nucleic acid fragment of (b) by further DNA or RNA fragments.

55. The process according to claim 54, characterized in that the DNA in step (c) is a PCR-amplified DNA fragment containing the human mitochondrial promoter of the light strand (P_L) as well as the gene for the mitochondrial transfer RNA leucine ($tRNALeu^{UUR)}$).

56. The process for the production of a chimerical peptide-nucleic acid fragment according to any one of claims 1 to 53, characterized by the following steps:

(a) optional extension of the nucleic acid containing a functional linkage group by further DNA or RNA fragments,

(b) reaction of the nucleic acid with functional linkage group or the extended nucleic acid of (a) with a linkage agent,

(c) reaction of the construct of (b) with amino acids at the carboxy-terminal end of a peptide containing a signal sequence, with the exception of a KDEL signal sequence.

57. The process according to claim 56, characterized in that the DNA in step (a) is a PCR-amplified DNA fragment containing the human mitochondrial promoter of the light strand (P_L) as well as the gene for the mitochondrial transfer RNA leucine ($tRNALeu^{UUR)}$).

58. Use of the chimerical peptide-nucleic acid fragment according to any one of claims 1 to 53 for the appropriate nucleic acid introduction into cell organelles and cells, characterized by reacting the fragment with cells or pretreated cell compartments.

59. Use according to claim 58, characterized in that the pretreated cell compartments are energized mitochondria.

60. Use of the chimerical peptide-nucleic acid fragment according to any one of claims 1 to 59 for the introduction into eukaryotic cells.

61. Use of a chimerical peptide-nucleic acid fragment according to claim 60, characterized by employing the 'particle gun' system, electroporation, microinjection or lipotransfection for the introduction into eukaryotic cells.